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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 78.WO1	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US2004/038339	International filing date *(day/month/year)* 15.11.2004	Priority date *(day/month/year)* 21.11.2003
International Patent Classification (IPC) or both national classification and IPC C12N15/12, C12N5/10, C07K14/705, G01N33/50		
Applicant ARENA PHARMACEUTICALS, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08.11.2005	20.02.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Huse, I Telephone No. +31 70 340-8951

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US2004/038339

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-40	as originally filed

Claims, Numbers

1-7, 8(part), 23-28	as originally filed
8(part), 9-22	received on 10.11.2005 with letter of 08.11.2005

Drawings, Sheets

1	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-15,21-28
	No:	Claims	16-20
Inventive step (IS)	Yes:	Claims	1-15,21-28
	No:	Claims	16-20
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations

   see separate sheet

1. Cited Documents

Reference is made to the following documents:

D1: WO 00/35274 A (THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE; REED, RANDALL, R; KRA), 22 June 2000

D2: HATT H ET AL: "CLONING, FUNCTIONAL EXPRESSION AND CHARACTERIZATION OF A HUMAN OLFACTORY RECEPTOR" CELLULAR AND MOLECULAR BIOLOGY, CMB ASSOCIATIONS, NOISY-LE-GRAND, FR, vol. 45, no. 3, 1999, pages 285-291

D3: BREER H ET AL: "EXPRESSION AND FUNCTIONAL ANALYSIS OF OLFACTORY RECEPTORS" ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, NEW YORK ACADEMY OF SCIENCES, NEW YORK, NY, US, vol. 855, 7 July 1997, pages 175-181

D4: KATADA SAYAKO ET AL: "Odorant response assays for a heterologously expressed olfactory receptor." BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. 13 JUN 2003, vol. 305, no. 4, 13 June 2003, pages 964-969

D5: KIEFER H ET AL: "Expression of an olfactory receptor in Escherichia coli: purification, reconstitution and ligand binding" BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 35, no. 50, 1996, pages 16077-16084

Re Item I

Basis of the report

The amendments filed with the letter dated 08-11-2005 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are claims 16, 18 and 19.

Page 19, lines 21-26 and page 20, lines 11-12 referred to by the applicant in the above letter, relate to methods of screening for **modulators** of an olfactory GPCR, i.e. compounds that increase or decrease the activity of the GPCR (cf. page 19, lines 13-16), and thus do not support the amendment of claims 16, 18 and 19. Pages 22-23 of the description, referring to methods for identifying **ligands** of an olfactory GPCR, only describe methods, wherein the binding of a potential ligand to the olfactory GPCR *per se*,

but not to a macroglial cell is assessed.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2 Novelty (Article 33(2) PCT)

The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 16-20 is not new in the sense of Article 33(2) PCT.

2.1 The claims refer to methods of screening for ligands of an olfactory GPCR and/or for olfactory modulators by contacting the olfactory GPCR with an agent. Document D1 discloses the heterologous expression of mouse olfactory receptors M4 and I-C6 as fusion proteins with the rhodopsin N-terminus in HEK-293 cells. The document further discloses the use of the transfected HEK-293 cells as screening system for identifying ligands/ odorants binding to the receptors (cf. pages 38-43). As a product is not rendered novel merely by the fact that it is produced by means of a new process (recombinant olfactory GPCR expressed in a macroglial cell), the olfactory receptors referred to in claims 16-20 can not be distinguished from the ones disclosed in document D1 and therefore claims 16-21 are not new (Article 33(2) PCT).

2.2 Claims 1-15 and 21-28 however are new in view of the prior art cited, as none of the documents discloses the heterologous expression of olfactory GPCRs in macroglial cells, as well as macroglial cells recombinantly expressing olfactory GPCRs and the use of said cells for screening modulators/ ligands/ odorants of the receptors.

3 Inventive Step (Article 33(3) PCT)

Moreover, the subject-matter of claims 1-15 and 21-28 is considered as involving an inventive step (Article 33(3) PCT) for the following reasons:

3.1 Document D1 represents the most relevant state of the art and is already described in detail above (cf. point 2.2).

3.2 The subject-matter of claims 1-15 and 21-28 differs in that olfactory receptors are heterologously expressed in macroglial cells, such as Schwann cells. The problem to be solved may therefore be regarded as providing a further expression system for the heterologous expression of olfactory GPCRs.

3.3 The solution, namely the use of macroglial cells, such as Schwann cells, is considered inventive in the sense of Article 33(3) PCT for the following reasons. The difficulty in functionally expressing olfactory GPCRs in heterologous cells, in particular because the receptors are often not translocated to the plasma membrane, is known in the art and addressed. Documents D1-D4 disclose the expression of olfactory receptors (mouse M4 and I-C6, human OR 17-40, mouse OR-EG) as fusion proteins with the rhodopsin N-terminus (cf. D1 and D4), or with the membrane import sequence of the 5-HT3 receptor (cf. D2) in various mammalian cells, such as HEK-293, COS-7 and CHO cells and the use of said cells for ligand screening (cf. D1, pages 38-43; D2, abstract; D3, abstract; D4, abstract). Document D3 moreover discloses the heterologous expression of olfactory receptors using a baculovirus/ Sf9 cell system and the functional analysis of the receptors by measuring cAMP and IP3 levels in response to various odorants (cf. abstract). Furthermore, document D5 discloses the expression of an olfactory receptor (OR5) in bacterial cells and ligand binding of the purified receptor (cf. abstract). The use of macroglial cells for heterologous expression of olfactory GPCRs however, is neither disclosed nor rendered obvious from the prior art cited. Moreover it would not have been expected, that the receptors are properly translocated to the cell membrane in said cell type and thus the cells can be used for ligand screening. The subject-matter of claims 1-15 and 21-28 is therefore considered inventive (Article 33(3) PCT).

contacting said cell with a candidate agent; and

assessing the effect of said candidate agent on an activity of said olfactory GPCR,

wherein a candidate agent that modulates an activity of said olfactory GPCR is a modulator of said olfactory GPCR.

9. The method of claim 7 or claim 8, wherein said agent is a small organic molecule.

10. The method of claim 7 or claim 8, wherein said agent is an odorant.

11. The method of claim 7 or claim 8, wherein said contacting is carried out in the presence of a known agonist of the olfactory GPCR.

12. The method of claim 7 or claim 8, wherein said modulator is selected from the group consisting of agonist, partial agonist, inverse agonist, and antagonist.

13. The method of claim 7 or claim 8, wherein said assessing is through the measurement of the level of GTPγS binding.

14. The method of claim 7 or claim 8, wherein said assessing is through the measurement of the level of a second messenger selected from the group of cyclic AMP (cAMP), cyclic GMP (cGMP), inositol 1,4,5-triphosphate (IP3), diacylglycerol (DAG), and Ca2+.

15. The method of claim 14, wherein said second messenger is cAMP.

16. A method of screening for a ligand of an olfactory GPCR, comprising:

producing said olfactory GPCR in a macroglial cell according to the method of claim 1;

contacting said macroglial cell with a candidate agent; and

assessing the binding of said candidate agent to said macroglial cell.

17. The method of claim 16, wherein said candidate agent is labeled.

18. A method of screening for a ligand of an olfactory GPCR, comprising:

producing said olfactory GPCR in a macroglial cell according to the method of claim 1;

contacting said macroglial cell with a candidate agent in the presence of a labeled known ligand of the olfactory GPCR; and

assessing the binding of said labeled known ligand of the olfactory GPCR;

wherein a decrease in binding of said labeled known ligand in the presence of said candidate agent is indicative of the candidate agent being a ligand of the olfactory GPCR.

19. A method of screening for an olfactory modulator, comprising:

producing a plurality of different olfactory GPCRs in different macroglial cells according to the method of claim 1, wherein each of said olfactory GPCRs is coupled to a G protein;

identifying a set of macroglial cells that are activated by a first agent, wherein said first agent is a known olfactory modulator;

contacting said set of macroglial cells with a second agent; and

assessing an effect of said second agent on an activity of the set of macroglial cells,

wherein a second agent that modulates the activity of one or more different cells of said set of macroglial cells activated by the first agent is an olfactory modulator.

20. The method of claim 19, wherein said set comprises three or more GPCRs.

21. A macroglial cell comprising a recombinant nucleic acid encoding an olfactory GPCR.

22. A kit comprising:

a macroglial cell; and

a nucleic acid encoding an olfactory GPCR.